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Materials

* 3x 1.5ml tubes per sample
* 10% bleach
* DI type II
* 70% ethanol
* Tube racks
* Sharpie
* Glass microscope slide
* Ruler (cm)
* Razorblade
* Glass pipette + bulb
* Tweezers
* Sedgewick rafter counting cell
* Kimwipes
* Compound microscope
* Camera + computer for microscope
* Dissecting microscope
* Drying oven
* Microscope -phone converter
* Phone to take photos
* ImageJ on a computer

Protocol

Sample Prep

1. Label 3x 1.5ml tubes per sample:
   1. Project name: “TL\_Octo”
   2. Sample ID
   3. Then either A, B, or C
2. Cut pieces of octocorals on a glass microscope slide using a razorblade.
3. Cut ¼ cm from the cut end of the octocoral and place into tube A.
4. Cut 1 cm from the cut end of the octocoral branch and place into tube B.
5. Freeze at -80˚C until ready for microscopy.

A circular object with different colored shapes

Description automatically generatedSclerites come in three forms:

Spindles (red) are larger and smoother than the other sclerites, they make up most of the internal structure

Capstans (yellow) are smaller but still uniform in shape, spiky sides

Clubs (blue) have a bulb shape on one end

**Sclerite lab protocol**

1. Set up computer on compound microscope with camera, open ToupView and make sure camera is connected.
2. Take out samples, in rounds of 8 because that’s how many fit in the vortex.
3. Add ~0.5ml of 10% bleach
4. A hand holding a plastic tip

   Description automatically generatedVortex on low-medium speed for 10 minutes or until chunk has completely broken down.
   1. There will be a protein core in the center, discard it.
5. Take sample off vortexer and allow sclerites to settle for a minute.
6. Pipette off the liquid and discard.
7. Add ~0.5ml DI and vortex again for ~60 seconds.
8. Pipette/dump/scrape sclerite material into a Sedgewick rafter counting slide.
   1. make sure sclerites are spread somewhat evenly using the pipette tip to spread them.
9. Spindle photos:
   1. A close-up of microscopic organisms

      Description automatically generatedUnder 40x magnification (red lens) position the focal point on one side of slide. Scroll horizontally until you reach one or more spindles.
   2. Spindles must not be broken, they should have two pointy ends.
   3. Take a photo using the “snap” button
   4. Continue scrolling horizontally and taking photos until you have photos of 10+ spindles.
   5. Once you have enough photos, go to file > Batch save and save all photos with the sample name in the file prefix.
   6. Return to the video feed, and right-click on the video tab > “close all but this”
10. A close-up of a microscope

    Description automatically generatedClub + Capstan photos:
    1. Under 100x magnification (yellow lens) position the focal point on one side of the slide.
    2. Scroll horizontally to find boxes with lots of clubs and capstans in them.
    3. Photograph 5 boxes (every other box along a single line).
    4. Make sure that one entire side of a box is in frame to use as a measuring tool.
    5. Once you have enough photos, go to file > Batch save and save all photos with the sample name in the file prefix.
    6. Return to the video feed, and right-click on the video tab > “close all but this”
11. Clean Sedgewick rafter, razorblade, glass slide, and pipette between samples using kimwipes and DI.
12. When finished for the day, clean all materials with fresh kimwipes and ethanol before putting away.

**Calice protocol**

1. Turn on oven to 50˚C.
2. Thaw B tubes and set up C tubes. You can do as many as you wish at a time.
3. Cover the octocoral piece with 10% bleach and rest for 10 minutes (do not vortex).
4. Use a pipette to remove bleach (discard).
5. Cover the octocoral piece with 10% bleach and rest for 10 minutes (do not vortex).
6. Use a pipette to remove bleach (discard).
7. Add DI to rinse off bleach.
8. Use tweezers to transfer skeleton to tube C.
9. Place open tube C in 50˚C oven for 5+ hours to dry. Sample is dry when it no longer accumulates moisture on the sides of the tube.
10. A close up of a cracker

    Description automatically generatedDry samples can be stored at room temp in tubes until future work is desired.
11. Set up microscope phone converter or camera on a *dissecting* microscope
12. Place dry samples on a glass slide.
13. Position ruler next to slide so that measurement markings are visible through the scope.
14. Photograph entire skeleton under magnification.
15. Roll sample over and take a second photograph (as a back-up).
16. Return to tube and store at room temp.

A screenshot of a computer

Description automatically generated**Photo Processing**

1. Load photos onto computer and open in imageJ.
2. For each new photo, set the scale
   1. Using the distance measuring tool, measure the scale distance (crosshairs on slide (1mm) or ruler (1cm))
   2. Click analyze > set scale… The pixel # should be filled in based on the distance you measured
   3. Enter 1 for “known value”
   4. Click Ok

A screenshot of a computer

Description automatically generated

1. Sclerite Size measurements
   1. For each photograph chose two clubs and two capstans to measure
   2. For each one, measure the length and width using the length tool
   3. For the spindle photos, measure length and width of each
   4. Repeat for 10 clubs, capstans, and spindles per sample.
   5. Manually enter all data into sheet. Copy only the “area” values.
2. A close up of a screen

   Description automatically generatedCalice size
   1. Use the counter tool to mark five calices to measure. To prevent bias, start at one end and move horizontally without skipping any.
   2. For each of the 5 calices, measure the height and width
   3. Enter data into data sheet
3. Calice density
   1. A close up of a cell

      Description automatically generatedUse the rectangular tool to highlight as much of the skeleton as possible.
   2. Enter the total area on the datasheet
   3. Use the counter tool or manually count every calice in the box. Only include individuals that have the absolute center within the box.
   4. Enter the count into the datasheet